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Genetics of Acheiropodia (the Handless and Footless Families of Brazil). VI. Formal Genetic Analysis

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Congenital amputations are frequently sporadic, and their etiologies are not well understood. The role of genetic factors is almost always unknown, and only occasionally do cases occur which follow a dominant (generally irregular) pattern of inheritance. Recessive cases are assumed to be exceptional. In this paper, acheiropodia will be shown to be one of these exceptional cases. Besides segregation analy sis, we will also discuss prevalence, gene frequency, population size, and number of founders.

Acheiropodia (Gr. *a* = absence; *cheir, cheiros* = hand; *pous, podos* = foot) is a congenital amputation mainly characterized by the complete absence of forearms, hands, and feet (fig. 1). Since the first report of acheiropodia was published in 1929 [1], and for several decades, only one Brazilian family with affected individuals has been known. This family has been widely quoted in papers and textbooks on medicine, biology, genetics, embryology, and pathology, generally with misinformation on the relationship between some normal and affected individuals and the etiology of the anomaly.

In a preliminary reanalysis of the original family, Koehler et al. [2] assumed recessive inheritance, showing that a dominant gene could not explain the data. After six new families were ascertained, Freire-Maia et al. [3] presented evidence favoring autosomal recessive inheritance and suggested that the mutation rate must be extremely low. Preliminary results have been published [4–7]. With the inclusion of two related sibships studied by Toledo and Saldanha [8] and the original family, as reanalyzed by Quelce-Salgado et al. [9], our present sample includes 22 sibships from six different Brazilian states [10].

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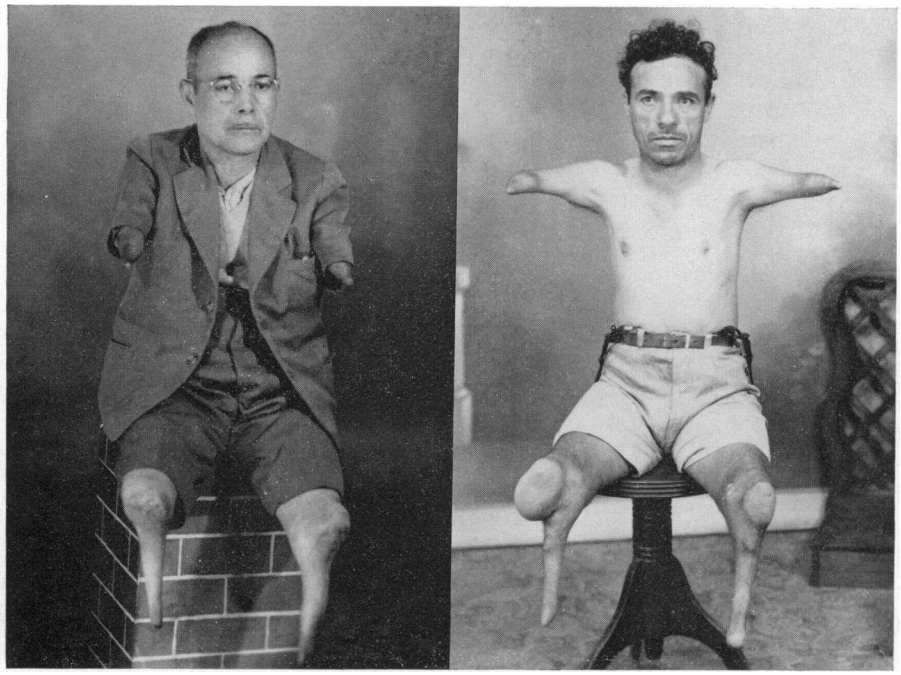

FIG. 1.—Acheiropods showing details of the malformation. Upper stumps of acheiropod at left were painted for dermatoglyphic analysis. The hypertrophy in knee of acheiropod at right was due to an accident.

The formal genetic analysis will be presented here (see also [11, 12]). Roentgenologic aspects were discussed by Koehler et al. [13], Quelce-Salgado et al. [9], and Toledo and Saldanha [8]. No chromosomal anomaly was found, and dermatoglyphic and laboratory analyses showed no relevant information [14].

SEGREGATION ANALYSIS

Segregation analysis [15–17] of the acheiropodia data was undertaken on a 16K CDC 3100 computer using the SEGRAN program written in FORTRAN IV. The data showed a segregation frequency of $p = .245 \pm .040$, which is close to the theoretically expected frequency of .25. In our sample, therefore, all affected individuals can be assumed to be the offspring of heterozygous parents. We can also conclude that acheiropodia is conditioned by an autosomal recessive gene in the homozygous state.

To estimate the proportion x of sporadic cases in the total population of cases, preliminary hand calculations were made. After the trial value of .05 was obtained, further computations revealed a final value not significantly different from zero. This is additional confirmation that acheiropodia is transmitted by an autosomal recessive gene. The possibility that occasionally other genetic or environmental mechanisms are operating cannot be excluded, but they did not occur in a detectable proportion of our cases.

A tentative value of $\pi = .60$ for the probability of ascertainment was obtained by hand calculations. This preliminary result was used in computer calculations, and the final value was $\pi = .55 \pm .07$. Thus in our case, there is a 55% probability of an affected individual being a proband, and consequently the ascertainment of the families was made through multiple incomplete selection.

PREVALENCE, GENE FREQUENCY, AND INCIDENCE

In the medical and scientific (including genetic) literature, the expressions prevalence and incidence are frequently misunderstood. Prevalence is the number of cases of a trait existing in a given area at a given time, and incidence refers to the frequency of new cases that appear in a particular period of time.

Given a fixed number of probands A through whom the families are ascertained in a finite population, prevalence n, and probability of ascertainment π, Barrai et al. [19] showed that $n = A/\pi$ and $\sigma_n = A\sigma_\pi/\pi^2$. Therefore, in our present case (16 living probands and $\pi = .55$), the prevalence of acheiropodia can be estimated as $16/.55 = 29$ (SE $= 16 \times .07/.55^2 \simeq 4$). This means approximately 29 cases of acheiropodia should exist in the population studied: we discovered 22. The total number of high risk cases is given by $n/(1 - x)$, and since $x = 0$, all 29 cases should be considered high risk. Assuming a population of 100 million for Brazil, a preliminary estimate of the frequency of acheiropodia would be of the order of $29/100{,}000{,}000 \simeq 3 \times 10^{-7}$.

The gene frequency has been estimated using the method proposed by Barrai et al. [19]. The estimates of α and σ were obtained from data published by Freire-Maia [20]. The gene frequency obtained from these estimates is $q = .0005$. By using maximum-likelihood scores, the most probable value of q was found to be $.0009 \pm .0005$. This estimate applies only to the population studied and should be accepted with caution.

Assuming $q = .0009$ and $\alpha = .0035$, the incidence of acheiropods at birth is $I = q^2 + \alpha q(1 - q) = .000004 = 4 \times 10^{-6}$ (roughly 1/250,000 births). The frequency of heterozygotes at birth is $2(1 - \alpha)q(1 - q) = .0018 = 0.18\%$, and the ratio of heterozygotes to affected is 450. Thus at birth the number of normal carriers of the acheiropodia gene could be 450 times larger than the number of affected. (If the gene frequency is smaller, the ratio will be larger.)

DISCUSSION

Because of the extreme rarity of the trait, the only practical method to study acheiropodia was through the ascertainment of affected individuals in the population. This sampling technique is generally known as incomplete selection [16]. The parents of an affected child are assumed to be heterozygous for the deleterious gene and have a genetic risk p. Due to chance alone, a number of heterozygous couples in the population do not have affected children and therefore are not ascertained, distorting the theoretically expected frequencies.

The number of nonascertained heterozygous couples in the population depends on the number of children (s) and equals p^s. The larger the value of s, the smaller

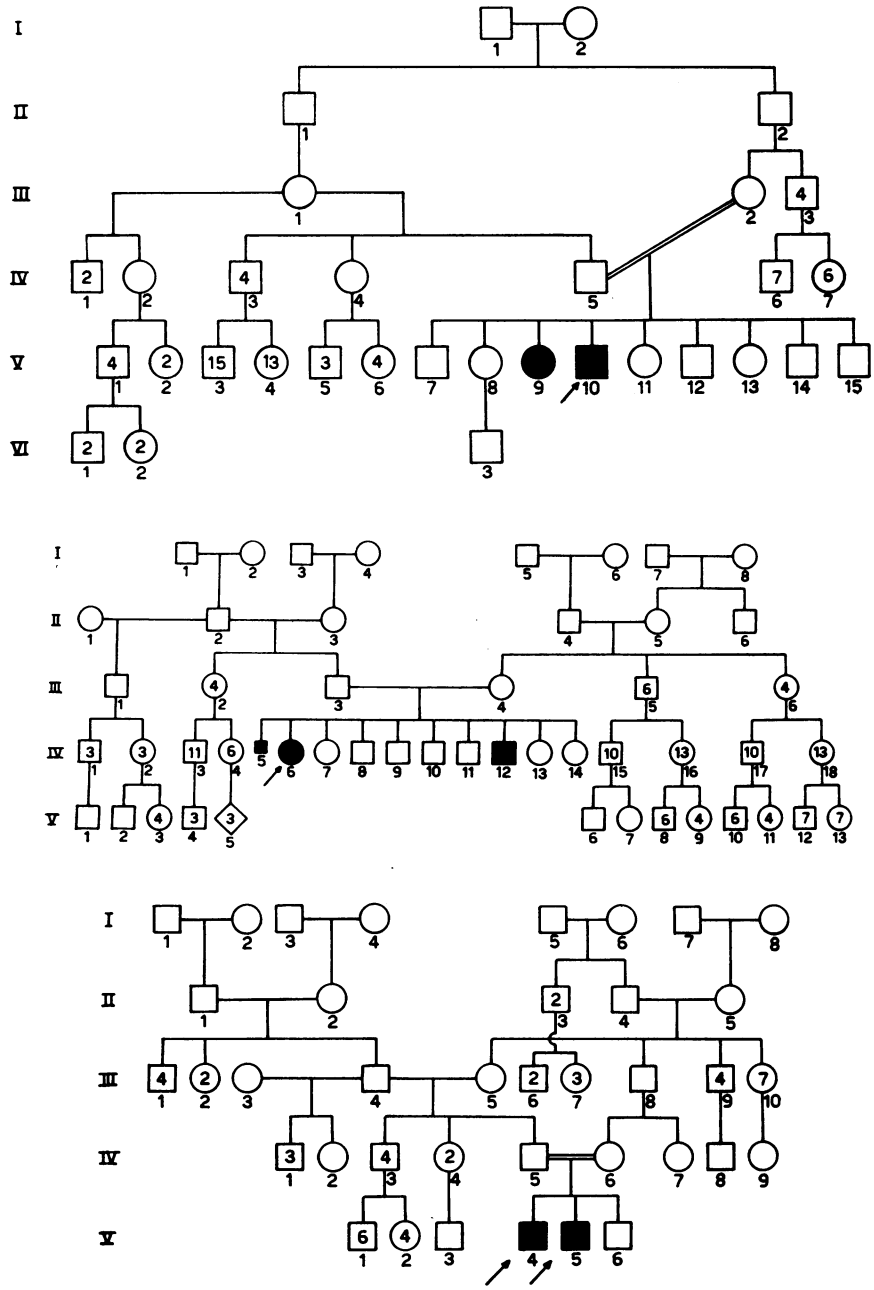

FIG. 2.—Pedigrees of acheiropodia: sibships 1 (*upper*), 10 (*middle*), and 11 (*lower*). Numbers inside symbols represent total number of individuals with these characteristics.

the number of heterozygous couples who will not be ascertained. When $s = 2$, 56% of the heterozygous couples will not be ascertained; but if $s = 20$, the frequency will be as low as 0.3%. Due to the relatively large value of s in the acheiropodia sample, the frequency of nonascertained heterozygous couples can be assumed to be relatively low, which could explain in part the good agreement between the uncorrected segregation frequency ($53/175 = 30.29\%$ of acheiropods) and the theoretical expectation of 25%.

Although the error introduced was relatively small, the method of ascertainment has changed the true segregation ratio, and more sophisticated methods of analysis had to be employed. As already shown [21], the acheiropodia data are compatible with the hypothesis of autosomal recessive inheritance, whether they are analyzed through a truncate incomplete selection model (Apert's a priori and the Haldane-Lejeune method) or through a simple incomplete selection model (Apert's a priori and Weinberg's sib method). Under the former model, the expected frequency of affected is .2785 ($\chi^2_1 = 0.36$, $P > .50$) and the corrected frequency, $.275 \pm .037$. Under the latter, the expected frequency of affected is .3405 ($\chi^2_1 = 1.35$; $P > .20$) and the corrected frequency, $.203 \pm .033$. The segregation analysis presented here confirms that the hypothesis of a rare autosomal recessive gene fits the data well.

Naturally other estimates (such as incidence) are subject to a series of assumptions which cannot be proved. For that reason we attempted to "directly" estimate the incidence. From census data of the Brazilian Institute of Geography and Statistics (IBGE; Rio de Janeiro), we estimated that approximately 6,900,000 births occurred in the same years and states as the acheiropod births. Because of incomplete registration of births in Brazil, particularly in some areas and some decades ago, this number must be viewed as an underestimate. Thus there is good agreement between the direct estimate of incidence ($49/6{,}900{,}000 = .000007 = 7 \times 10^{-6}$)* and the indirect estimate (4×10^{-6}). Clearly, these estimates must be accepted with caution and refer specifically to the population under analysis.

When the population size is known, it is possible to use the prevalence to calculate the frequency of those affected at birth and then to estimate gene frequency and mutation rate. If population size is unknown (as in our case), it can be estimated from prevalence and incidence. Incidence (I) is the ratio between the actual number of cases (n) and population size [19]. Therefore, $N = n/I = 29/(4 \times 10^{-6}) = 7{,}250{,}000$, where N is an underestimate of the size of the population where acheiropods were ascertained. This underestimate is due to the assumption that acheiropods have the same viability as normal individuals from the general population. We calculated that, when compared to their normal sibs, only about 72% of the acheiropods ever born were represented (alive) in the population.† Therefore, a less biased estimate of population size would be given by $N = 29/(4 \times 10^{-6} \times 0.72) \simeq 10{,}000{,}000$, or roughly 10% of the assumed Brazilian

* At the time the IBGE data were obtained we had information on only 49 acheiropods.

† Of 53 acheiropods born, 22 (42%) were alive at the time of the survey compared to 71 (58%) of their 123 sibs. Thus in relation to their normal sibs, only 72% of the acheiropods have survived.

population. As usual, this estimate is subject to error of a size difficult to evaluate.

Due to the extreme rarity of the anomaly and to its occurrence (as far as we know) only among Portuguese descendants in Brazil, an attractive hypothesis is to ascribe all cases of acheiropodia, or at least all the Brazilian cases, to the same mutant gene. According to this hypothesis, all the families presented here should be somehow related (although we failed in showing such relationship). After such a hypothesized unique mutation occurred, the first heterozygous individual was born.* Therefore, the size (N_0) of the original population where such an individual was born can be estimated as $N_0 = 1/2q = 556$. If acheiropodia (in Brazil, at least) originated from a single surviving mutant individual (or, roughly speaking, a single mutation), the number of founders could have been of the order of 500 individuals. A discussion of this hypothesis is in press [22].

SUMMARY

A genetic analysis is presented of data from 22 Brazilian sibships with cases of acheiropodia (the handless and footless families of Brazil). Segregation analysis performed using a 16K CDC 3100 computer showed a segregation frequency of .245 ± .040, which is close to the expected value of .25. No sporadic cases were detected. The ascertainment of the probands was through multiple incomplete selection ($\pi = .55 \pm .07$). The data are consistent with the hypothesis of an extremely rare autosomal recessive gene as the etiological factor in acheiropodia.

Prevalence is estimated as 29 ± 4, which is the same as the number of high risk cases; gene frequency equals .0009 ± .0005, and the incidence at birth is 4×10^{-6} by the indirect method or 7×10^{-6} by the direct method. The frequency of heterozygotes at birth is assumed to be 0.18% (450 times the frequency of affected). Population size is approximately 10 million, and the number of founders on a unique-mutation hypothesis is estimated as about 500. All these estimates are first approximations and must be accepted with caution.

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